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Comparison of screening methods for antibiotics in beef kidney juice and serum☆

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ARTICLE INFO

Article history:
Received 19 June 2008
Received in revised form
4 August 2008
Accepted 11 August 2008
Published on line 19 August 2008

Keywords:
Antibiotics
Screening
Microbial inhibition assays
Liquid chromatography–mass spectrometry

ABSTRACT

Rapid screening tests can be used as part of an efficient program designed to monitor veterinary drug residues in cattle. In this work, three rapid tests designed to screen samples for the presence of antibiotic residues, the Fast Antimicrobial Screen Test (FAST), Premi® and Kidney Inhibition Swab (KIS™) tests, were compared using beef kidney juice and serum samples. In order to provide a realistic assessment, potentially incurred samples of beef kidney juice and serum were obtained from 235 carcasses which had been retained by inspectors in a processing plant for further testing. In addition, liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis was conducted on these samples to identify what antibiotics were present, if any, and their levels. The comparison of the three rapid screening test results with those from LC–MS/MS analysis allowed for a more complete comparison of the relative sensitivity of these analytical methods, as well as valuable information on false positive and negative response rates.

Published by Elsevier B.V.

1. Introduction

The use of antibiotics in food animals has generated concern due to the potential for increasing antimicrobial resistance, in addition to hypersensitivity for some individuals. Tolerance limits for antibiotic residues or maximum residue limits (MRLs) have been set around the world, and agencies such as the U.S. Food Safety and Inspection Service (FSIS) monitor the food supply to ensure that antibiotic residue concentrations do not exceed these levels. Methods are available for determination of antibiotic residues in a variety of matrices, but many of these methods are relatively expensive and time consuming. As the great majority of samples tested do not contain violative antibiotic residue levels [1], screening methods are increasingly being used to rapidly identify the few samples which would need further testing, allowing monitoring programs to operate more efficiently.

FSIS currently uses the Fast Antimicrobial Screen Test (FAST) as a screening method for antibiotic residues in animal tissues. It can be performed in processing plants by trained inspectors and is rapid and easily interpreted. Additional microbial inhibition-based screening methods have become available and we recently compared the FAST with two others, the Premi® test and the Kidney Inhibition Swab (KIS™) test, examining the detectability of eight selected antibiotics by these kits in beef kidney juice and serum samples for-

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0003-2670/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aca.2008.08.005

tified over a range of concentrations [2]. While studies of fortified samples are an important part of evaluating new methods, ultimately such methods must be studied using actual incurred samples. Our intent in this work was, thus, to use the same three methods to screen potentially incurred samples obtained from a beef processing plant. Comparison of the responses of each of the three rapid tests to these samples would provide valuable data for evaluative purposes; however, this approach would not be sufficient, in itself. Positive or negative responses from the rapid tests would need to be correlated with an actual presence or absence of antibiotic residues in the samples in order to establish their reliability.

Liquid chromatography (LC)–mass spectrometry (MS)/MS methods have been developed to identify and quantitate a great variety of veterinary drug residues in a variety of matrices. These methods can be invaluable in providing confirmation for specific residues present in a sample. The great majority of LC–MS/MS methods are designed for analysis of a single drug residue, or for several members of one class of veterinary drugs in a given matrix [3,4]. LC–MS/MS is a relatively expensive approach, and sample preparation methods are not always rapid. For this technique to be used effectively to screen samples for antibiotic residues, a large number of analytes from a range of different classes of drugs must be rapidly extractable from samples and analyzed via one or two injections on the instrument. Multiclass, multiresidue LC–MS/MS methods are now beginning to become available [5–8]. We have been developing such a method ourselves, capable of determining >100 veterinary drugs, which utilizes a rapid and simple sample preparation. In this work, we used this multiclass, multiresidue LC–MS/MS approach to analyze the same potentially incurred samples which had been analyzed using the three rapid screening tests for the occurrence and levels of antibiotics present. The LC–MS/MS results allowed us to establish which samples contained what antibiotic residues, and the levels which were present. Comparison of these results with those from the rapid screening tests allowed us to better determine their actual efficacy and their propensity for false positive and false violative results.

2. Experimental

2.1. Chemicals and materials

Norfloxacin, ofloxacin, lomefloxacin·HCl, oxolinic acid, sulfaguanidine, sulfanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, sulfisoxazole, sulfadimethoxine, sulfaphenazole, sulfasalazine, carbadox, virginiamycin, oxacillin sodium salt monohydrate, dicloxacillin sodium monohydrate, minocycline·HCl, oxytetracycline·HCl, chlortetracycline·HCl, doxycycline·HCl, hygromycin B, apramycin sulfate, and tobramycin were obtained from Sigma (St. Louis, MO, USA). Ciprofloxacin, sulfadoxine, lincomycin·HCl, tilmicosin, erythromycin, tylosin, amoxicillin, cephapirin sodium, ampicillin sodium, cephalexin, cefazolin, penicillin G sodium, cloxacillin sodium, nafcillin sodium, penicillin V, spectinomycin·HCl, streptomycin sulfate, dihydrostreptomycin sulfate, amikacin, kanamycin sulfate, gentamicin and neomycin sulfate were obtained from United States Pharmacopeia (USP, Rockville, MD, USA). Danofloxacin, pirlimycin·HCl, and desfurylceftiofur cysteine disulfide (DCCD) were obtained from Pfizer (Groton, CT, USA). Florfenicol amine was obtained from Schering and orbifloxacin was provided by Schering Plough (Kenilworth, NJ, USA). Sarafloxacin·HCl and difloxacin·HCl were provided by Abbott (North Chicago, IL, USA). Desethylene ciprofloxacin and enrofloxacin were provided by Bayer (Merriam, KS, USA). $^{13}C_6$-Sulfamethazine was from Cambridge Isotopes (Andover, MA, USA), sulfaquinoxaline was from Pfaltz & Bauer (Waterbury, CT, USA), desacetyl cephapirin sodium was from AFSSA (Fougeres, France) and tetracycline·HCl was from Acros (Geel, Belgium). Sulfabromomethazine was synthesized in house.

Individual antibiotic stock solutions (1000–2000 $\mu g\,mL^{-1}$) were prepared in acetonitrile or methanol, except for the β-lactams and aminoglycosides, which were prepared in water. Composite working standard solutions ($\leq 10\,\mu g\,mL^{-1}$) were prepared containing all studied antibiotics except the β-lactams and aminoglycosides (added separately). All stock and working standard solutions were stored at −20 °C.

Materials for the FAST test (agar plates, neomycin standard disks, *Bacillus megaterium* spore suspension) were supplied by the Food Safety and Inspection Service (FSIS) Midwestern Laboratory (St. Louis, MO, USA). Premi® test materials (vials and pre-treatment solution) were obtained from DSM (Geleen, The Netherlands). KIS™ materials (vials and feed extraction buffer) were provided by Charm Sciences (Lawrence, MA, USA). Supplies for these three tests were stored at 4 °C, except for solutions of the feed extraction buffer (−20 °C).

Acetonitrile, methanol, acetic acid, and 40 μm C_{18} prep LC packing were from JT Baker (Phillipsburg, NJ, USA). Potassium dihydrogen phosphate, ethylene diamine tetraacetic acid (EDTA) disodium salt dihydrate, heptafluorobutryic acid (HFBA), formic acid and trichloroacetic acid were from Sigma. All aqueous solutions used deionized water prepared with an E-pure system (Barnstead, Dubuque, IA, USA).

2.2. Preparation of kidney juice and serum samples

Control kidneys and blood samples (from steer carcasses) and potentially incurred samples (from inspector-retained carcasses [9]) were obtained at a local processing plant. Blood samples were allowed to clot and then centrifuged (1500 × *g*, 20 min) and decanted to produce serum, which was then stored, in aliquots, at −20 °C. Kidneys were immediately frozen, and then later thawed to produce kidney juice, which was centrifuged (3000 × *g*, 10 min) and the supernatant stored, in aliquots, at −20 °C.

2.3. FAST, Premi® and KIS™ screening procedure

Kidney juice and serum samples from any potentially incurred carcass were analyzed simultaneously using these three tests. Aliquots of the samples to be tested, as well as aliquots of control kidney juice and serum (negative controls) were thawed, kept on ice, and used for the three tests.

FAST. FAST agar plates were streaked with *B. megaterium* spores and then 2–3 blank 6 mm paper disks (Becton Dickinson & Co., Sparks, MD, USA) as well as a standard neomycin disk (positive control) were placed on each plate. Kidney juice or serum samples to be analyzed (25 μL) were immediately pipetted onto the blank disks. Plates were then incubated 7 h at 44 °C, after which zones of inhibition of microbial growth were measured with a ruler. A positive response was determined as a zone size ≥10 mm, based on zone measurement variations surrounding negative control disks.

Premi® and KIS™ tests. Both Premi® and KIS™ tests use an indicator to detect pH change as a measure of growth inhibition of *Bacillus stearothermophilus*. There are minor differences between the tests in terms of vial design, protocol, and indicators used. Kidney juice or serum samples (100 μL) were pipetted into the Premi® or KIS™ vials and incubated in a 64 °C heater block until the negative controls turned from purple to yellow in color (2.75–3.5 h, depending on test and lot). The Premi® test required pre-heating of kidney juice vials at 80 °C (10 min) to inactivate lysozyme-like interferents, prior to the 64 °C incubation. Both Premi® and KIS™ tests required a pre-treatment of serum samples (100 μL sample + 33 μL pre-treatment solution, or 100 μL sample + 100 μL feed extraction buffer, respectively) prior to pipetting the pre-treated sample into the vial. Both tests relied upon a visual examination to determine a positive (blue/purple color) or negative (yellow/green color) result.

2.4. *Extraction of veterinary drugs for LC–MS/MS analysis*

2.4.1. *Aminoglycosides*

Kidney juice or serum (1.0 mL) was twice shaken vigorously or vortex mixed (5 min) with 10 mM KH_2PO_4, 0.4 mM EDTA, 2% TCA (5 mL). After centrifugation (3716 × *g*, 10 min), the pH of the combined supernatants was adjusted to 7.4–8.0 with aqueous NaOH. Extracts were loaded onto BakerBond solid phase extraction (SPE) CBX 500 mg/6 mL columns which had been preconditioned with methanol (5 mL) and then water (5 mL). After washing with pH 7.4 water (5 mL), and drying of the column, aminoglycosides were eluted with 10% acetic acid in methanol (3 mL). The eluate was evaporated to dryness at 40 °C under N_2 (Zymark TurboVap LV, Hopkinton MA, USA), 20 μg mL^{-1} tobramycin was added (50 μL) and then the volume was adjusted to 1 mL with 5 mM HFBA.

2.4.2. *Other antibiotics*

The majority of antibiotics were extracted following an approach used recently for β-lactams [10]. Kidney juice or serum samples (1.0 mL), to which had been added 5 μg mL^{-1} $^{13}C_6$-sulfamethazine (40 μL), were shaken for 5 min with 4 mL of either acetonitrile:water (4:1, kidney juice) or acetonitrile (serum). Samples were then centrifuged (3716 × *g*, 5 min) and the supernatants decanted into a disposable centrifuge tube containing C_{18} (250 mg) for a technique known as dispersive SPE. The tubes were vortexed briefly and then shaken for 30 s. After centrifugation (3716 × *g*, 5 min), an aliqout (2.5 mL) of supernatant was transferred to a 15 mL glass centrifuge tube and the samples evaporated to <0.5 mL volume under N_2 at 40 °C using a TurboVap LV. Volumes were then adjusted to 1.0 mL with water and 600 μL portions of each sample were transferred to 0.45 μm Mini Uni-prep PVDF filter vials (Whatman, Florham Park, NJ, USA).

2.5. *LC–MS/MS*

LC–MS/MS used an Agilent 1100 HPLC system, including a degasser, binary pump, autosampler and column heater, linked to either a Thermo Finnigan LCQ Deca ion trap mass spectrometer (aminoglycosides screen) or a Applied Biosystems Sciex API 3000 triple quadrupole mass spectrometer (other antibiotics screen and all quantitation). Chromatography involved a Phenomenex Prodigy ODS-3 (5 μm, 150 mm × 3.0 mm) column, with a Phenomenex ODS (4.0 mm × 2.0 mm) Security Guard column. For aminoglycosides, a gradient composed of 5 mM aqueous HFBA (A) and 5 mM methanolic HFBA (B) was used: 0–0.5 min (5% B), 1.0 min (40% B), 9–12.5 min (80% B) and 14.5–24.5 min (5% B). For the other antibiotics, a gradient composed of 0.1% aqueous formic acid (A) and 0.1% formic acid in acetonitrile (B) was used: 0 min (2% B), 15.0–19.0 min (100% B). With both instruments, column eluate was diverted to waste at the beginning and end of the chromatographic runs to minimize contamination of the MS source. The column was maintained at 30 °C and the flow rate was 0.3 mL min^{-1}. Both mass spectrometers were operated in positive ion electrospray mode. Parent and product ions, along with retention times, are listed for all antibiotics in Table 1. All other parameters such as collision energy and Q for the ion trap, and declustering potential, focusing potential, collision energy, and collision exit potential for the triple quadrupole were optimized for each analyte. An initial extraction and screening analysis were performed on all serum and kidney juice samples using both the aminoglycoside method and the other antibiotics method (1 MS/MS transition monitored). Along with the samples to be tested on a given day, a fortified sample containing all analytes and a corresponding matrix matched sample were analyzed for quality control purposes. Concentrations for these quality control samples were either 10, 25, 50, or 100 ng mL^{-1} for the triple quadrupole instrument, or 0.5, 1.0 or 2.0 μg mL^{-1} for aminoglycosides on the ion trap instrument. Samples indicating the possible presence of an antibiotic were retested using two transitions for the triple quadrupole instrument along with additional calibration curve samples to provide confirmatory and quantitative data.

3. Results and discussion

This comparison study was set up so that kidney juice and serum samples from a given carcass would be tested simultaneously with the FAST, Premi® and KIS™ tests. Screening of a separate aliquot of each sample for aminoglycosides and the other antibiotics using LC–MS/MS was conducted in a separate experiment. For any samples which appeared to contain antibiotic residues by LC–MS/MS, another aliquot of the same sample was reanalyzed to provide quantitation, using two transitions with the triple quadrupole instrument. While the FAST and KIS™ tests are typically run using swabs which had been inserted into kidney tissue to absorb juice, we elected to produce juice from the entire kidney and use

Table 1 – LC–MS/MS parameters and quality control results for aminoglycosides (A) and other antibiotics (B)[a]

Method	Group	Analyte	$[M+H]^+$ (m/z)	Retention time (min)	Product ions (m/z)	Kidney juice % recovery (% R.S.D.)	Serum % recovery (% R.S.D.)
A	Aminoglycosides	Spectinomycin	333	9.2	189, 140, 227	75 (17)	69 (32)
		Hygromycin B	528	8.6	352, 177, 321	94 (23)	90 (27)
		Streptomycin	582	9.6	263, 407, 540	88 (13)	92 (12)
		Dihydrostreptomycin	584	9.6	263, 409, 542	91 (12)	93 (13)
		Amikacin	586	9.9	425, 324, 485	87 (15)	85 (14)
		Kanamycin	485	10.1	324, 163, 366	86 (9)	86 (12)
		Apramycin	540	10.6	378, 344, 361	88 (13)	83 (13)
		Gentamicin	478	11.0	322, 160, 205	87 (17)	82 (17)
		Neomycin	615	11.0	455, 293, 323	80 (11)	83 (16)
B	β-Lactams	Desacetyl cephapirin	382	7.2	152, 226	184 (34)	92 (18)
		Amoxicillin	366	7.8	349, 114	72 (69)	112 (101)
		DCCD	549	8.4	183, 241	106 (17)	50 (26)
		Cephapirin	424	8.4	292, 152	–	89 (29)
		Ampicillin	350	8.9	106, 192	106 (11)	107 (26)
		Cephalexin	348	8.9	158, 174	92 (13)	92 (20)
		Cefazolin	455	10.6	323, 156	111 (15)	96 (17)
		Penicillin G	335	13.2	160, 176	110 (9)	120 (16)
		Oxacillin	402	14.2	160, 243	101 (16)	104 (8)
		Cloxacillin	436	14.7	277, 160	108 (10)	101 (9)
		Naficillin	415	14.8	199, 171	101 (29)	109 (12)
		Dicloxacillin	470	15.4	160, 311	106 (14)	101 (12)
	Macrolides	Tilmicosin	870	10.0	697, 174	61 (28)	20 (49)
		Erythromycin A	735	10.8	158, 576	86 (27)	36 (47)
		Tylosin	917	11.0	174, 773	91 (31)	79 (25)
	Quinolones	Desethyl ciprofloxacin	306	8.8	288, 268	94 (22)	62 (22)
		Norfloxacin	320	9.0	276, 302	79 (22)	55 (28)
		Ofloxacin	362	9.0	318, 261	78 (31)	62 (27)
		Ciprofloxacin	332	9.1	288, 314	86 (20)	59 (27)
		Danofloxacin	358	9.2	340, 314	60 (23)	55 (39)
		Lomefloxacin	353	9.2	237, 309	72 (40)	58 (43)
		Enrofloxacin	360	9.3	316, 245	63 (32)	55 (35)
		Orbifloxacin	396	9.4	352, 295	97 (20)	96 (11)
		Sarafloxacin	386	9.6	368, 342	75 (24)	81 (31)
		Difloxacin	400	9.7	356, 299	58 (27)	67 (35)
		Oxolinic acid	262	12.3	244, 216	91 (24)	91 (22)
	Sulfonamides	Sulfaguanidine	215	5.4	156, 108	108 (22)	108 (11)
		Sulfanilamide	173	6.4	156, 92	82 (44)	88 (47)
		Sulfacetamide	215	9.3	156, 108	108 (10)	107 (13)
		Sulfadiazine	251	9.6	156, 92	112 (16)	110 (12)
		Sulfathiazole	256	9.7	156, 108	108 (13)	114 (17)
		Sulfapyridine	250	9.9	156, 108	109 (15)	112 (14)

Table 1 (*Continued*)

Method	Group	Analyte	$[M+H]^+$ (*m*/*z*)	Retention time (min)	Product ions (*m*/*z*)	Kidney juice % recovery (% R.S.D.)	Serum % recovery (% R.S.D.)
		Sulfamerazine	265	10.3	156, 108	108 (10)	111 (13)
		Sulfamethazine	279	10.7	186, 124	111 (12)	114 (12)
		Sulfamethizole	271	10.7	156, 108	112 (15)	112 (14)
		Sulfamethoxypyridazine	281	10.8	156, 108	109 (14)	114 (13)
		Sulfachloropyridazine	285	11.7	156, 108	111 (17)	113 (13)
		Sulfaethoxypyridazine	295	11.9	156, 108	110 (17)	110 (12)
		Sulfadoxine	311	11.9	156, 92	111 (14)	109 (12)
		Sulfamethoxazole	254	12.1	156, 108	112 (17)	113 (11)
		Sulfisoxazole	268	12.3	156, 113	114 (15)	108 (12)
		Sulfaquinoxaline	301	12.7	156, 108	107 (24)	114 (11)
		Sulfadimethoxine	311	12.8	156, 108	113 (19)	113 (12)
		Sulfaphenazole	315	12.9	160, 159	113 (18)	111 (12)
		Sulfabromomethazine	359	14.0	156, 108	104 (19)	112 (12)
		Sulfasalazine	399	14.1	381, 119	111 (21)	115 (16)
	Tetracyclines	Minocycline	458	8.6	441, 352	55 (33)	32 (36)
		Oxytetracycline	461	9.2	426, 443	89 (20)	41 (19)
		Tetracycline	445	9.4	410, 427	74 (43)	58 (28)
		Chlortetracycline	479	10.2	462, 444	72 (27)	85 (67)
		Doxycycline	445	10.3	428, 410	68 (16)	66 (37)
	Other	Florfenicol amine	248	5.1	230, 130	101 (16)	104 (11)
		Lincomycin	407	8.4	126, 359	118 (21)	109 (19)
		Carbodox	263	9.7	231, 130	84 (22)	101 (24)
		Pirlimycin	411	9.8	112, 363	110 (27)	81 (18)
		Virginiamycin	526	13.8	508, 109	102 (34)	76 (41)

[a] $n = 16$, with outliers removed.

Table 2 – Results of kidney juice (KJ) and serum (S) screening[a]

Sample no.	FAST		Premi®		KIS™		LC–MS/MS			
	KJ	S	KJ	S	KJ	S	KJ antibiotic	Concentration[b] ($ng\,mL^{-1}$)	Serum antibiotic	Concentration ($ng\,mL^{-1}$)
1				+					Gentamicin	8
									Kanamycin	1
6	+						Dihydrostreptomycin	1,100		
42							Dihydrostreptomycin	41		
129							Dihydrostreptomycin	39		
194							Dihydrostreptomycin	48		
195							Dihydrostreptomycin	91		
212							Dihydrostreptomycin	6		
15							Penicillin G	2	Penicillin G	1
21			+		+		Penicillin G	3	Penicillin G	det
196									Penicillin G	2
22							Oxytetracycline	14		
23							Oxytetracycline	3		
26				+			Oxytetracycline	54	Oxytetracycline	3
49							Oxytetracycline	16		
55				+			Oxytetracycline	5		
70							Oxytetracycline	19		
102							Oxytetracycline	2		
191							Oxytetracycline	33		
17				+			DCCD	22		
28							DCCD	5		
48						+	DCCD	28		
59							DCCD	9		
67							DCCD	10		
90							DCCD	12		
130							DCCD	6		
142							DCCD	2		
96							Floramphenicol amine	33		
38							Pirlimycin	2		
198			+				Pirlimycin	29		
203			+				Pirlimycin	22		
95									Sulfadimethoxine	2
171									Sulfadimethoxine	1
11	+		+		+		Penicillin G	3	Penicillin G	2
							Dihydrostreptomycin	14,000	Dihydrostreptomycin	288
							Streptomycin	75		
65							Oxytetracycline	2		
							Lincomycin	4		
							Sulfamethazine	2		
100	+		+		+		Oxytetracycline	17	Oxytetracycline	2
							Dihydrostreptomycin	7,200	Sulfadimethoxine	<1
									Sulfamethazine	1
104			+		+		Pirlimycin	22		
							DCCD	2		
188				+			Oxytetracycline	4		
							DCCD	12		
228			+			+	Oxytetracycline	57	Penicillin G	1
							Pirlimycin	17	Oxytetracycline	2
229			+	+	+	+	Sulfamethazine	141	Sulfamethazine	233
							Oxytetracycline	4		

det: detected, not quantitated.

[a] Absence of a "+" or an antibiotic name designates a negative assay response.

[b] U.S. tolerances ($ng\,g^{-1}$) in kidney (unless otherwise specified): dihydrostreptomycin (2000), penicillin G (50), oxytetracycline (12,000), DCCD (2000 for desfurylceftiofur), florfenicol amine (3700 in liver), pirlimycin (500 in liver), streptomycin (2000), lincomycin (none) and sulfamethazine (100).

Table 3 – Summary of positive results from kidney juice (KJ) and serum (S) screening

Test	Detected level					
	>U.S. tolerance[a]		<U.S. tolerance[a]		False positives[b]	
	KJ	S	KJ	S	KJ	S
FAST	2	0	1	0	2	1
Premi®	3	1	5	1	1	30
KIS™	3	1	2	1	13	11

[a] U.S. tolerance in kidney.
[b] Positive test, but negative LC–MS/MS result.

this directly, with the goal of obtaining a more representative and uniform sample for the varied screening and LC–MS/MS analyses.

The three rapid screening tests were easily performed. The FAST was easily interpreted, with zones of inhibition simply measured with a ruler. Although we elected to read the results after 7 h, the zones of inhibition could be measured anytime between 6 and 18 h, adding to the test's flexibility. For the even more rapid Premi® and KIS™ tests, changes in color were sometimes difficult to interpret, which may be reflected in the number of false positive results obtained. In addition, incubation time for the Premi® and KIS™ tests appeared to be quite important, as continued incubation at 64 °C past the time at which a negative control turned yellow in color could lead, eventually, to positive samples turning yellow (negative), as well.

The LC–MS/MS method for the majority of antibiotics worked very well. The extraction procedure was quite rapid and simple to carry out, facilitating analysis of the large number of samples. Quality control recoveries covering the range of 10–100 $ng\,mL^{-1}$ (Table 1) were very good for most analytes in each matrix. Notable exceptions include cephapirin in kidney juice, its conversion to desacetylcephapirin being a well known phenomenon [11]. Most analytes provided reasonable R.S.Ds, although a few exceptions (e.g., amoxicillin) provided highly variable results across this range of concentrations. The LC–MS/MS method for the aminoglycosides worked well, although the process was more time consuming due to the cartridge-based SPE clean up. Quality control recoveries and R.S.Ds for these analytes were also very reasonable (Table 1).

One of the first observations to be made from this study was that the great majority of carcasses sampled (196 out of 235) were not found to contain any antibiotic residues by LC–MS/MS. Results from the carcasses which did contain antibiotic residues, along with the corresponding responses of the three rapid screening tests, are listed in Table 2. A summary of results for the three rapid screening tests is presented in Table 3.

The ideal screening test would only respond positively to a sample when an analyte present is banned, or if the levels of an analyte present are greater than the tolerance. In this manner, violative samples would be detected, without needing to further reanalyze non-problematic samples. In this study, LC–MS/MS analysis identified three carcasses having levels of an analyte greater than the U.S. tolerance in kidney. All three rapid screening tests successfully detected the two samples containing high levels of dihydrostreptomycin in kidney juice. The Premi® and KIS™ tests identified a sample with sulfamethazine above the U.S. tolerance for kidney (both kidney juice and serum), as well, while the FAST did not.

LC–MS/MS detected three antibiotics having no listed U.S. tolerance in beef. Of these, in serum, the Premi® detected a sample containing <10 $ng\,mL^{-1}$ gentamicin and a trace of kanamycin. None of the tests detected a kidney juice sample containing <5 $ng\,mL^{-1}$ lincomycin.

The FAST provided the fewest false positives (positive test, no antibiotic detected by LC–MS/MS), and appeared to be, in general, less sensitive than the other two screening tests for the antibiotics detected in these samples. We had found FAST to be similarly less sensitive in our earlier study with fortified samples of 8 selected antibiotics, although none of these screening tests had provided the desired sensitivity for all analytes tested [2]. KIS™ and Premi® both had a significant number of false positives in this study, with all but one for the Premi® in serum, while the KIS™ shows them distributed between kidney and serum. The reason for this disparity is

Table 4 – Kidney juice and tissue analysis

Sample no.	Antibiotic	Kidney juice thaw 1 concentration ($ng\,mL^{-1}$)	Kidney juice thaw 2 concentration ($ng\,mL^{-1}$)	Kidney tissue concentration ($ng\,g^{-1}$)
11	Dihydrostreptomycin	14,000	15,000	17,000
100	Dihydrostreptomycin	7,200	5,800	11,000
21	Penicillin G	3	3	0
191	Oxytetracycline	33	38	62
198	Pirlimycin	29	17	66
48	DCCD	28	20	15
67	DCCD	10	9	6
229	Sulfamethazine	141	171	236

unclear, but it suggests kidney juice may be a significantly better matrix for Premi®.

A potential point to consider in this work is that one or more of the false positive responses provided by the rapid screening tests may actually be a true positive, representing an uninvestigated antibiotic or metabolite. While the list of antibiotics included in the LC–MS/MS analysis is quite lengthy, inclusion of all antibiotics known to exist is not a practical option. Furthermore, the possibility of an antibiotic metabolite triggering a positive response from the rapid screening tests is a subject which would require additional study. Although one should be aware of the above issues, LC–MS/MS remains the standard for antibiotic residue analysis.

Considering samples containing antibiotics at levels below U.S. tolerance in kidney, the FAST detected a sample containing 1 μg mL^{-1} dihydrostreptomycin, but no others. The Premi® detected four samples with <30 ng mL^{-1} pirlimycin (tolerance available only in liver), two of which also had low levels of other drugs, and two samples with <10 ng mL^{-1} penicillin G or oxytetracycline. The KIS™ detected two samples containing <10 ng mL^{-1} penicillin G, one of which had a low level of oxytetracycline, and one with pirlimycin (<25 ng mL^{-1}) and a very low level of DCCD.

Several samples were found to contain mixtures of antibiotics. In such cases, LC–MS/MS provides a significant advantage, for identification and quantitation, as mixtures can be problematic to interpret with microbial inhibition screening assays.

In this study we planned to compare kidney juice and serum as matrices for screening. A point of interest regarding serum as a matrix is that the volume obtained from a given volume of blood was found to be highly variable, apparently depending on the hydration state of the animal. Thus serum provided a potentially more variable sampling matrix than had been expected, although it could still provide a good matrix for antemortem screening, given more consistently hydrated animals. Kidney juice for these studies was prepared by freeze/thawing kidneys. We found that additional kidney juice could be obtained by a second cycle of freeze/thawing. For a few selected carcasses, we compared the levels of antibiotic found in first thaw kidney juice, second thaw kidney juice, and the remaining kidney tissue, to see if there were substantial differences resulting from these treatments. The results are shown in Table 4. Two samples containing dihydrostreptomycin contained similar levels in the first and second thaw juice samples, as well as in the remaining tissue. A sample containing oxytetracycline and one containing pirlimycin contained lower levels in the two juice samples, but then higher levels in the remaining tissue. Two samples containing DCCD showed slight decreases in going from the first to second thaw juice and then to tissue, whereas a sample containing sulfamethazine showed the opposite trend. Although further study is clearly needed, these preliminary results suggest that relative distribution between the kidney juice and tissue may be analyte dependent.

4. Conclusions

The three rapid screening tests were found to be easily performed and could be useful for in-plant preliminary screening of samples, although no one of the three tests is likely to be a perfect match for the needs of a broad antibiotic sampling program. LC–MS/MS provides a much more definitive method for detection and identification of antibiotic residues. With a rapid sample preparation method, it can be a very useful part of a monitoring program, although it is not as well suited for field-based screening.

Acknowledgments

We acknowledge Dr. Ann Predgen, Mark Uhlrich, and Limei Yun for their technical assistance, Charm Sciences for KIS™ test kits, DSM for discounted Premi® test kits, Bayer for desethyl ciprofloxacin, Schering for orbifloxacin, and Pfizer for samples of danofloxacin and DCCD. We also thank Dr. Eric Verdon (AFSSA) for providing a sample of deacetylcephapirin.

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